

ELECTRODE POTENTIALS OF BIOLOGICALLY
IMPORTANT OXIDATION-REDUCTION
SYSTEMS WITH UNSTABLE
OXIDANTS

STUDIES ON OXIDATION-REDUCTION. XX

EPINEPHRINE AND RELATED COMPOUNDS

A DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE
JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH
A REQUIREMENT FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BY

TUNG-TOU CHEN

Baltimore, 1933

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STUDIES ON OXIDATION-REDUCTION*

XX. EPINEPHRINE AND RELATED COMPOUNDS†

TUNG-TOU CHEN‡

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(Received for publication, July 24, 1933)

INTRODUCTION

Earlier papers in this series have been concerned largely with oxidation-reduction indicators. The value of such indicators as an aid to the determination of the generalized intensity factor of oxidation-reduction processes within living cells has been ably demonstrated by Cohen, Chambers, and Reznikoff (9). However, before chemical interpretations of the so called "oxidation-reduction potential" of living cells can be made with confidence there must be more definite knowledge of specific oxidation-reduction systems known to occur in living tissues. Among the small number of these systems which are amenable to direct potentiometric study are those in which certain, special derivatives of ortho- and paradihydroxybenzene are the reductants. The occurrence of these derivatives both in animal and plant cells is wide-spread. Some occur as the anomalous products of animal metabolism; others as hormones or poisons. In plants they have

* Previous papers of this series (Papers I to XVI) were published in the *Public Health Reports, United States Public Health Service*, and (Papers XVII to XVIII) in the *Journal of the American Chemical Society*. Paper XIX is in press in the *Journal of the American Chemical Society*.

† A preliminary report was made before The Fourteenth International Physiological Congress at Rome, 1932 (see *Sunti comunicaz. scient., XIV cong. internaz. fisiol.*, Rome, 21 (1932)).

‡ Fellow of Peiping Union Medical College.

TABLE I
 Compounds Studied

Name	Donor or source	Description*
Benzohydroquinone	Eastman Kodak Company	M.p. 169.5°
Benzoquinone	" " "	Steam-distilled, m.p. 110-111°
Catechol	" " "	Original sample, m.p. 102-103°; 3 times recrystallized, m.p. 102-103°
Pyrogallol	" " "	
Gallic acid	" " "	
Gentisinic acid	Kahlbaum	Once recrystallized, m.p. 202-203°
Protocatechuic acid	"	Once recrystallized, m.p. 200°
Ethyl ester of gentisinic acid	Drs. L. Hellerman and M. E. Howard	M.p. 77.5-78°; prepared from Kahlbaum's gentisinic acid
Ethyl ester of protocatechuic acid	" "	M.p. 131.5-132°; prepared from Kahlbaum's protocatechuic acid
3,4-Dihydroxyphenylalanine	Hoffman-La Roche, Inc.	(1) Racemic, synthetic; (2) levo, from <i>Vicia faba</i> ; once recrystallized, melting with decomposition at 275°
Epinephrine (bitartrate)	H. A. Metz Laboratories, Inc.	(1) Levo $[\alpha]_D^{26} = -51^\circ$; (2) dextro $[\alpha]_D^{26} = +63^\circ$. Both synthetic
Epinephrine (free base)	Parke, Davis and Company	Levo, from beef adrenals
" "	Dr. J. J. Abel	" " <i>Bufo agua</i>
Norhomoepinephrine	Dr. W. H. Hartung (Sharp and Dohme)	
Epinine	Dr. C. S. Leonard (Burroughs Wellcome and Company)	
Adrenalone	H. A. Metz Laboratories, Inc.	

* All melting points are uncorrected.

been associated with oxidative catalysis and with mechanisms of defense against infection. Some of them have been identified as intermediates in the formation of pigments.

It is of particular interest that the oxidants of practically all these compounds are unstable, especially in solutions having pH values within the physiological range and above. Therefore, if these compounds are to be protected from a virtually destructive oxidation in those natural environments which are reputed to be the seats of vigorous oxidative processes, the intensity level at which the general oxidative processes proceed must be below the level required for the oxidation of these specific substances. Accordingly data for the characteristic potentials of the systems of which these naturally occurring derivatives of catechol and benzohydroquinone are the reductants would help in defining the level of oxidation-reduction intensity of the living tissue in which they are found.

The systems which we have studied are indicated by the names of their respective reductants. These are listed with certain details in Table I. Included in this group are several synthetic compounds of pharmacological or structural interest.

Procedure

If an oxidation-reduction system have an unstable component, such changes may occur during the course of an ordinary titration as to make the interpretation of electrode potentials difficult or even impossible. Some success has been attained by quick titrations, as, for example, those of Clark, Cohen, and Gibbs (8) or by extrapolation methods such as were employed by Biilmann and Blom (3). Fieser (14) made discontinuous titrations with extrapolations of the time-potential curves and obtained satisfactory data for several unstable systems including that of catechol. However, these procedures were found to be inadequate for the purposes which are to be described. Accordingly Ball and Clark (2) adapted to electrode potential measurements the principle of rapid mixing and of observation of the flowing mixture; a procedure first employed for liquids by Hartridge and Roughton (17) and used by Schmidt (26), Saal (25), and Dirken and Mook (12) for the study of various aspects of ionic reaction.

The scarcity of certain of the substances precluded the use of

large volumes of solution and consequently made impracticable an apparatus of the type employed by previous workers. A diagram of the apparatus used, which was constructed entirely of glass, is shown in Fig. 1.

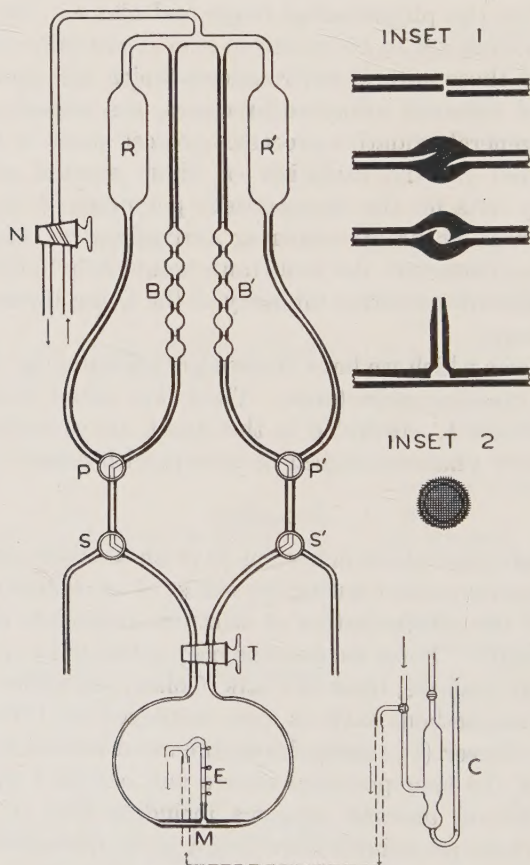


FIG. 1. Mixing apparatus and cell. *R* and *R'* are reservoirs; *B*, *B'*, series of small bulbs; *P*, *P'*, *S*, *S'*, *T*, *N*, stop-cocks; *M*, mixing chamber; *E*, electrode chamber; *C*, calomel half-cell. Inset 1 shows construction of mixing chamber, *M*; Inset 2, platinum gauze sealed between sections of glass tubing. The diameter of the mixing chamber is 5 mm., depth 3 mm., inlets 0.15 mm., outlet 1 mm. The diameter of the electrode chamber is 3 to 4 mm., length 8 cm. The rate of flow of the mixture is approximately 20 ml. per minute.

Buffered solutions of the reductant (*e.g.* epinephrine) and an oxidizing agent (*e.g.* $\text{Ce}(\text{SO}_4)_2$) are stored in the 150 ml. reservoirs, *R* and *R'* respectively. Each of the series of five, small bulbs, *B* and *B'*, can be filled from their respective reservoirs through the stop-cocks *P*, *P'*. The volume of each individual bulb is accurately calibrated; each holds about 2 ml. Before making a run, each set of bulbs is filled to the uppermost calibration mark and connected to the stop-cock *T* through the stop-cocks *P*, *S* and *P'*, *S'* as shown. A stop-watch is started at the instant that stop-cock *T* is turned. There is then simultaneous flow of both solutions through the already filled system under a constant pressure (10 cm. of Hg) of purified nitrogen gas. This pressure, from a 12 liter reservoir, is applied through one arm of the stop-cock *N*. The two solutions meet at the mixing chamber, *M*, are thoroughly mixed, and pass to electrode chamber *E* attached by means of a ground glass joint. The mixture in passing through this chamber bathes each of a series of electrodes with a solution which is presumably of a fixed composition at any one electrode. After flowing past the last electrode, the mixture forms a flowing, liquid junction with a solution saturated with potassium chloride. The latter solution comes from a reservoir and is in direct connection with a saturated KCl calomel half-cell marked *C*. The potentials of the electrodes (usually four) and the time required for the emptying of any convenient number of bulbs of each set are measured. The ratio of oxidant to reductant at the moment of mixing can be calculated from the concentrations and the relative rates of flow of the two original solutions. This ratio can be varied by changing the concentrations of the solutions, or, as in the more usual case, by altering the setting of either stop-cock *S* or *S'*. Approximate adjustments of these settings are aided by pointers attached to the stop-cock keys. The time required for a given cross-section of the mixture to pass from the orifices in *M* to any electrode in *E* is calculated from the values for the rates of flow mentioned above and the calibrated space between the orifices in *M* and the given electrode. These data are employed in the extrapolation of the observed potentials to zero time and will be discussed more fully later.

The most important feature is the mixing chamber, the construction of which is illustrated by Inset 1, Fig. 1. A capillary

tube of Pyrex glass is broken and its ends placed in abutment but mutually offset. During fusion of the junction in a flame, a slight pressure is exerted so that, as the walls of the capillary contract, their ends are turned away from one another. A hole is then ground perpendicular to the capillaries and centered between their contracted ends. This is cautiously widened until it makes contact with the ends of the capillaries. The latter then open tangentially into this hole, which is the mixing chamber. Both inlet orifices should have about the same diameter. The interior of this chamber is ground smooth and then an outlet is fused on. This outlet carries the male end of a ground glass joint for connection to the female end of the electrode chamber.

The electrodes are of 80 mesh, platinum gauze sealed between sections of glass tubing (Inset 2, Fig. 1). Contact is made by hooking platinum leads to platinum wire which has been wound around the protruding edges of each gauze electrode. The electrodes are freshly plated with gold each time before use. Gold-plated electrodes come to equilibrium with the solutions more rapidly than blank platinum.

The whole apparatus is enclosed in a stirred, air thermostat. Most measurements were made at 30°. All potentials here recorded have been brought to the hydrogen standard in accordance with the conventions of Clark (6). In the tables, E'_0 will designate the potential of the system at 50 per cent reduction and a specified value of pH and E_0 the potential of the system at 50 per cent reduction extrapolated to pH = 0.

Corrections were made for change of potential attributable to those changes of pH which are caused by the products formed in the oxidation-reduction process. Such corrections and minor manipulative procedures will not be described, since they involve numerous details and are similar to those described in previous papers of this series. It was necessary to substitute veronal buffers as described by Michaelis (20) for the pH range covered by the borate system. Borates cannot be used with polyhydroxy compounds.

Direct tests for completeness of mixture and reliability of potentials were made by applying the procedure to the comparatively stable system of which benzohydroquinone is the reductant. Table II shows the results of one of several series of

such tests. Readings at all three electrodes agreed within 0.2 millivolt; the average is reported. The column headed "Running" gives the potentials observed with the mixture flowing past the electrodes, while that headed "Standing" gives the potentials which were observed after the flow had been stopped and the mixture allowed to stand in contact with the electrodes for several minutes. The agreement between the two sets of readings and between these readings and measurements taken in the ordinary way indicates that the mixing and reaction were practically com-

TABLE II

Hydroquinone (0.002 M) Versus Quinone (0.002 M)

pH 4.654; temperature $30.0^\circ \pm 0.05^\circ$; E'_0 (theoretical) = 0.4158. Assumed values, $E_0 = 0.6955$; $-\Delta E'_0/\Delta \text{pH} = 0.0601$.

Reduction	0.03006 log [S _r]/[S _o]	E _h observed*		E' ₀	
		Running	Standing	Running	Standing
<i>per cent</i>					
22.04	-0.0165	+0.4321	+0.4323	+0.4156	+0.4158
36.09	-0.0075	0.4233		0.4158	
43.35	-0.0035	0.4193	0.4192	0.4158	0.4157
55.09	+0.0027	0.4133	0.4133	0.4160	0.4160
55.25	0.0027	0.4132	0.4133	0.4159	0.4160
62.86	0.0069	0.4090	0.4093	0.4159	0.4162
71.35	0.0119	0.4040	0.4042	0.4159	0.4161
80.97	0.0189	0.3971	0.3974	0.4160	0.4163
Average.....				0.4159	0.4160
By usual procedure.....				0.4158	

* Average of potentials at three electrodes.

plete before any given cross-section of the solution reached the first electrode and that there is no significant effect introduced by the streaming of the mixture past the electrodes. The largest deviation, 0.3 millivolt, is equivalent to a difference of about 0.5 per cent in the composition of the mixture. In general, deviations are small when the delivery from each jet is approximately the same. Variations in the rate of flow of the KCl solution had no detected effect. It is important to note that essentially similar results were obtained when, instead of mixing quinone and hydroquinone, hydroquinone was mixed with an oxidizing agent.

When we are dealing with a system, one component of which is unstable, we find the change of composition reflected in the change of potential from electrode to electrode. Assume that the mixing is complete before a given cross-section reaches the first electrode and that, after mixing, the solution changes its composition with time in accordance with some law which is operating during mixing. Then, if the rate of flow be uniform, the composition of the solution at any one electrode will be constant, even though the composition at one electrode differ from that at another electrode. Accordingly, and if the preliminary period of flow has been adequate, the lag in the adjustment of electrode potential to changing composition should be eliminated as it is not in the ordinary procedure of quick titrations. Consequently if there can be found a uniform relation between the potentials at the several electrodes and the time at which a given cross-section of the flowing solution reaches each electrode, it should be possible to estimate by extrapolation the potential for the initial, undecomposed mixture.

In the majority of cases the observed relation was

$$-dE_h/dt = C \quad (1)$$

Equation 1 may be derived from simple assumptions. Let the hydrion activity and ionic strength of a solution be constant. Then Equation 2 and its derivative, Equation 3, may be applied.

$$E_h = E'_0 + \frac{RT}{nF} \ln \frac{[S_o]_t}{[S_r]_t} \quad (2)$$

$$\frac{dE_h}{dt} = \frac{RT}{nF} \frac{d[S_o]_t}{[S_o]_t dt} - \frac{RT}{nF} \frac{d[S_r]_t}{[S_r]_t dt} \quad (3)$$

$[S_o]_t$ and $[S_r]_t$ are the molar concentrations of total oxidant and total reductant at time t . During mixing and reaction both $[S_o]$ and $[S_r]$ vary with time. Let these processes be complete at one of the first and subsequent electrodes. Then $[S_r]_t$ is a constant which may be calculated from the initial compositions of the unmixed solutions. Also $d[S_r] = 0$. Hence

$$\frac{dE_h}{dt} = \frac{RT}{nF} \frac{d[S_o]_t}{[S_o]_t dt} \quad (4)$$

Let the oxidant decompose in accordance with a pseudo first order reaction

$$\frac{-d[S_o]_t}{dt} = k[S_o]_t \quad (5)$$

Equations 4 and 5 give Equation 1. The experimental verification of Equation 1 is presumptive evidence of the validity of Equation 5. This has been found in the cases studied by Biilmann and Blom (3), by Phillips, Clark, and Cohen (23), and by Fieser (14). If the point giving the data for the first electrode falls upon the straight line passing through the other points, it is presumptive evidence that Equation 5 is applicable at the time of the first point and consequently that mixing and reaction had been practically complete before this time. Hence the potential corresponding to the ratio $[S_o]/[S_r]$, calculated as if there were no decomposition of oxidant, must be appreciably higher than that of the first electrode. If the line be extrapolated to zero time, the potential at the intercept would be that of a solution formed by instantaneous mixing and reaction. Since this cannot be the actual case, the potential at this intercept must be too high. Therefore the desired value should be somewhat higher than the potential at the first electrode and somewhat lower than the potential at the intercept.

Having no definite information about the course of the mixing and reaction, we have arbitrarily extrapolated to the zero time in each instance. Since the time for the first point was usually about 0.2 second and since there are given in Tables VI to X the values of $-\Delta E_h/\Delta t$ in millivolts per second, the reader may see that about one-fifth the value of $-\Delta E_h/\Delta t$ in any instance is somewhat *more than the maximum possible error* attributable to the theoretical difficulties discussed above.

In a complete analysis consideration should be given to the following. As an imagined cross-sectional surface of the mixture advances through the narrow electrode chamber, it should suffer distortion because of the friction at the walls. Hartridge and Roughton (17) have pointed out that this factor is minimized by the rotational motion imparted to the mixture as it is formed in the mixing chamber; but we have no information as to how far this type of flow will persist after passing a gauze electrode. There-

fore a gauze electrode placed perpendicular to the wall may not be exposed to a solution of strictly uniform composition. The observed electrode potential will probably be that of the portion containing the largest percentage of undecomposed oxidant and this probably constitutes the greater part of the advancing section. However, since refined corrections to obtain precisely by extrapolation the true potential cannot be made, the matter under discussion needs no further consideration now.

TABLE III
Catechol (0.002 M) Oxidized with Ceric Sulfate (0.002 N). In 0.1 M Acetate Buffer
pH 4.388; temperature $30.0^\circ \pm 0.05^\circ$.

Reduction	$0.03006 \log \frac{[S_r]}{[S_o]}$	E_h^* observed	E'_0	$E'_0 \dagger$ corrected
<i>per cent</i>				
15.14	-0.0225	+0.5520	+0.5295	+0.5289
22.99	-0.0158	0.5457	0.5299	0.5293
24.95	-0.0144	0.5443	0.5299	0.5293
39.38	-0.0056	0.5362	0.5306	0.5301
53.39	+0.0018	0.5281	0.5299	0.5294
59.77	0.0052	0.5248	0.5300	0.5295
59.99	0.0053	0.5251	0.5304	0.5299
74.99	0.0143	0.5167	0.5310	0.5306
80.83	0.0188	0.5116	0.5304	0.5300
86.18	0.0239	0.5064	0.5303	0.5300
Average.....				0.5297
$0.06011 \times \text{pH}.....$				0.2637
$E_0.....$				0.7934

* Average of potentials at four electrodes.

† Corrected for acidity change due to oxidation-reduction process.

It would be desirable to extend the observations to include a time nearer that of the first contact between corresponding cross-sections of the initial solutions, since it might be possible, in certain instances, to obtain points on the chart closer to the potential axis at zero time. However, several of our observations indicate that, while an improved apparatus might accomplish this, difficulties may be encountered in the inherent slowness of certain of the oxidation processes. When unused oxidizing agent remains, there should be a competition between the two systems in their

effects upon the electrodes and this will seriously complicate interpretations. This competition seems evident in some of the results of Saal (25) who applied the principle of Hartridge and Roughton to the study of a variety of oxidation processes and who was able to make observations corresponding to 0.004 second after mixing.

In Tables III and IV are typical results testing the relation of Equation 2, where n has been regarded as 2. Although other systems with more unstable oxidants did not yield data agreeing

TABLE IV

Epinephrine (0.002 M) Oxidized with Ceric Sulfate (0.002 N). In 0.5 M Sulfuric Acid

pH = 0.286; temperature $30.0^\circ \pm 0.05^\circ$.

Reduction	$0.03006 \log [S_r]/[S_o]$	E_A^* observed	E'_0
<i>per cent</i>			
28.51	-0.0119	+0.8030	+0.7911
30.48	-0.0108	0.8027	0.7919
38.94	-0.0059	0.7969	0.7910
47.82	-0.0011	0.7916	0.7905
56.06	+0.0032	0.7864	0.7896
60.14	0.0054	0.7850	0.7904
81.88	0.0197	0.7697	0.7894
Average.....			0.7906
$0.06011 \times \text{pH}$			0.0172
E_0			0.8078

* Average of potentials at four electrodes.

as well as do the data for catechol and epinephrine, there has been found no occasion to use any value for n other than 2.

All the cases studied should fall within Group A, Class 2 of the outline given by Clark and Cohen (7). The full equation, stated with numerical coefficient for 30° , is

$$E_h = E_o + 0.03006 \log \frac{[S_o]}{[S_r]} + 0.03006 \log [K_{r_1}K_{r_2} + K_{r_1}(\text{H}^+) + (\text{H}^+)^2] \quad (6)$$

Here, in addition to symbols previously defined, (H^+) is the hydron activity, and K_{r_1} and K_{r_2} are the apparent dissociation constants of those phenolic groups of the reductant which are

destroyed by the oxidation of the hydroquinone to the quinone. The dissociation constants must be defined as apparent because in the derivation of Equation 6 we have assumed constant ionic strength and solutions in which the buffer salts predominate.

Since several of the systems contain components having carboxyl or other acidic or basic groups not directly affected by the oxidation-reduction process but subject to change in strength by the operation of this process, the constants for these groups should doubtless be included in the equation. As noted by Clark and Cohen (7) and as illustrated in many subsequent experimental studies, such changes cause displacement of the curve relating E'_0 to pH. Some cases in which such displacements are suspected will be noted later. In no case do the experimental data permit accurate values to be assigned to the dissociation constants of these groups. Accordingly Equation 6 will suffice for the description of the results.

In most cases the instability of the oxidant increased so greatly as (H^+) decreased that it was impracticable to make measurements at values of (H^+) sufficiently low to make K_{r_1} and K_{r_2} significant in Equation 6. Hence most of the data are described by the simplified equation

$$E_h = E_0 + 0.03006 \log \frac{[S_o]}{[S_r]} - 0.0601 \text{ pH} \quad (7)$$

The case of catechol is peculiar and will receive special attention later.

Results

Table III contains the results of a typical experiment with catechol. The uniformity of the E'_0 values obtained over the range 15 to 86 per cent reduction is satisfactory. In solutions of the indicated pH the oxidant of catechol decomposes so rapidly that within 6 minutes after its formation nearly 50 per cent would have disappeared. Under such conditions the difficulty of obtaining a titration curve by the usual procedure can readily be appreciated. Yet in this experiment the rate of decomposition of the oxidant was relatively so slow that the potentials read at the four electrodes agreed within 0.1 millivolt and extrapolation was unneces-

sary. Such was also the case at all pH values less than 4.4¹ and not until the alkaline range was reached was extrapolation necessary.

An example of the results obtained with epinephrine is shown in Table IV. The oxidant of epinephrine is so unstable that it was only in the acid region that good agreement was obtained over a wide range of the titration curve. As pH increases, the rate of decomposition of the oxidant increases rapidly and it is necessary to resort to extrapolation of potentials in solutions with pH values greater than 2. In general, extrapolation of potentials was not employed when the value for $-\Delta E_h/\Delta t$ given in Tables VI to X was less than 0.1 millivolt per second. In Table V are the potentials obtained with several different preparations of epinephrine. Three of these are from widely different sources; yet no

TABLE V

Values for Several Preparations of Epinephrine Oxidized with Ceric Sulfate
Temperature $30.0^\circ \pm 0.05^\circ$.

Sample and origin	pH	E'_0	E_0
<i>l</i> -Synthetic; Metz.....	0.286	+0.791	+0.808
Beef glands; Parke, Davis.....	0.286	0.790	0.807
<i>Bufo agua</i> *.....	0.286	0.791	0.808
<i>l</i> -Synthetic; Metz; $[\alpha]_D^{25} = -51^\circ$	1.282	0.734	0.811
<i>d</i> -Synthetic; Metz $[\alpha]_D^{25} = +63^\circ$	1.282	0.735	0.812

* A sample kindly supplied by Dr. Abel.

significant difference in potential was found. No significant difference in potential was observed for the systems of which *l*- and *d*-epinephrine are the reductants.

Available space will not permit the presentation of all the data in the detail shown in Tables III and IV. In the majority of cases the data fit the theoretical equation satisfactorily. Therefore the results may be summarized. Each value of E'_0 given in Tables VI to IX represents the average of at least four determinations at different percentages of reduction. In all cases a 0.002

¹ Ceric sulfate can be used as an oxidizing agent at this pH only in acetate buffers. The usual rapid hydrolysis of ceric sulfate at this pH is retarded by the presence of the acetate ions; its oxidizing ability remains unaltered.

TABLE VI

Catechol and Epinephrine. Relation of E'_0 and of $\Delta E_h/\Delta t$ to pH
 Temperature 30.0°.

Oxidizing agent	pH	Catechol			Epinephrine		
		E'_0	E_0 calculated assuming $-\frac{\Delta E'_0}{\Delta \text{pH}} = 0.0601$	$-\frac{\Delta E_h}{\Delta t}$	E'_0	E_0 calculated assuming $-\frac{\Delta E'_0}{\Delta \text{pH}} = 0.0601$	$-\frac{\Delta E_h}{\Delta t}$
				<i>mv. per sec.</i>			<i>mv. per sec.</i>
Ce(SO ₄) ₂	0.286	0.772	0.789	0.033	0.791	0.808	0.006
"	0.906	0.737	0.791	0.010	0.755	0.809	0.02
"	1.280	0.714	0.791	0.011	0.733	0.809	0.03
Cl ₂	1.390	0.707	0.791				
Ce(SO ₄) ₂	1.530	0.700	0.792	0.010	0.718	0.810	0.04
"	1.810	0.680	0.788	0.007	0.699	0.808	0.08
Br ₂	2.680	0.628	0.789	0.005	0.645	0.806	0.42
Ce(SO ₄) ₂	3.237	0.594	0.788		0.609	0.804	2.0
Br ₂	3.420				0.600	0.806	5.5
"	4.074	0.547	0.792	0.011	0.556	0.801	15.0
Ce(SO ₄) ₂	4.388	0.530	0.793	0.025			
Br ₂	4.654	0.511	0.791	0.014			
Cl ₂	4.654	0.514	0.791	0.018			
Br ₂	4.815				0.515	0.804	25.0
Ce(SO ₄) ₂	5.031	0.489	0.792	0.027			
Br ₂	5.241				0.497	0.812	90.0
"	6.079	0.427	0.793	0.040	0.430	0.796	30.0
Cl ₂	6.083	0.428	0.793				
Br ₂	6.828	0.385	0.795	0.16	0.390	0.800	30.0
K ₃ Fe(CN) ₆	7.660	0.333	0.794	1.0	0.345	0.805	150.0
"	8.520	0.286		3.8			
"	8.549*	0.283		4.1			
"	9.276	0.253		9.0			
"	9.690*	0.239		17.5			
"	10.486*	0.198		29.2			
"	10.489	0.199		29.8			
"	10.910*	0.177					
"	11.291*	0.153		38.0			
"	11.661*	0.135		48.0			
"	12.068*	0.111		38.2			
"	12.528*	0.092					
"	12.997*	0.052		39.6			
Average.....			0.792			0.809 (first 5 values)	

* Catechol dissolved in conductivity water; oxidizing agent dissolved in buffer solution (see text).

M solution of the reductant and a 0.002 N solution of the oxidizing agent were employed.

Table VI contains a summary of the E'_0 values for both catechol and epinephrine. The E_0 values in both cases are calculated by assuming $-\Delta E'_0/\Delta \text{pH} = 0.0601$ at 30° . The average of the E_0 values obtained in the acid range places the normal potential of catechol at 0.792 volt for 30° . Ball and Clark (2) reported a normal potential of 0.798 volt for this system at 23° . The normal potential measured at 37.4° was lower than that at 30° by a corresponding amount. Fieser and Peters (15) reported the normal potential for this system at 25° to be 0.794 and 0.789 when the solvents were 0.5 N H_2SO_4 and 1.0 N H_2SO_4 respectively. These values are somewhat lower than those reported here when temperature differences are considered. The decrease in normal potential with increasing acidity noted by these workers is evident to a less degree in our results. We are inclined to attribute this effect to a change in the liquid junction potential with the more acid solution. We have found no occasion to employ a correction factor for an association between the oxidant and the reductant, as did Fieser and Peters. These workers do not state the concentration of catechol in their experiments, so no comparison is possible.

The highest pH value at which epinephrine could be studied with any satisfactory results was 7.66. Even here the potentials are not quite reliable. As indicated in Fig. 2, the values of E'_0 for the higher values of pH depart from the relation $-\Delta E'_0/\Delta \text{pH} = 0.0601$. This may be due to errors resulting from the great instability of the oxidant of the epinephrine system, although it might be due to a shift in the dissociation exponent of the substituted amino group incident to oxidation of the catechol group. Since the potentials observed with distinctly acid solutions are the more reliable, we may average these. On this basis the normal potential of the epinephrine system is 0.809 at 30.0° . The preliminary value reported by Ball and Clark (2) is undoubtedly too low.

Making due allowances for experimental errors, we find no indication that the value of the normal potential has been influenced by the type of oxidizing agent used. But it is interesting to note that chlorine acts much more slowly than does bromine.

Indeed, it was impracticable to use chlorine with epinephrine because the decomposition of the oxidant kept pace with the oxidation process. The same was true of ceric sulfate in acetate buffer. Such difficulties did not arise with catechol since its

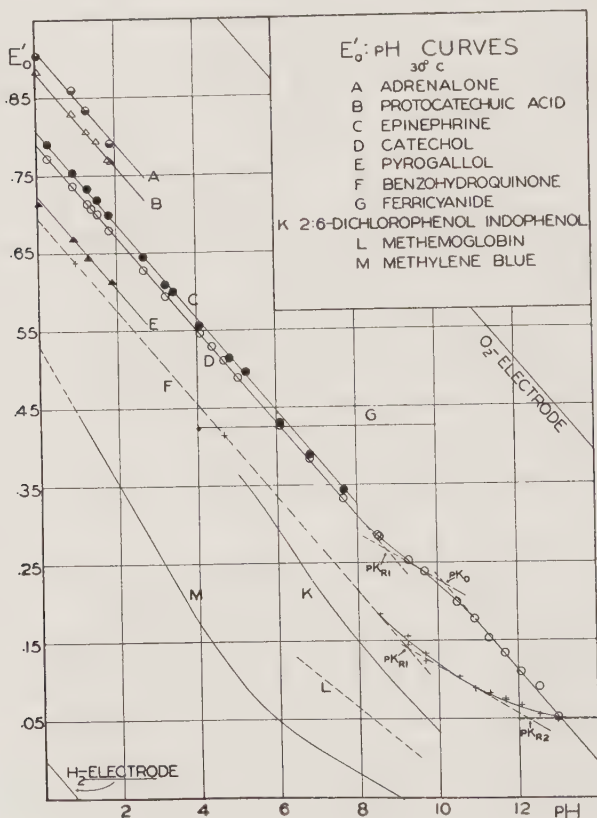


FIG. 2. Relation of E'_0 to pH

oxidant decomposed much more slowly than the oxidant of epinephrine.

The behavior of the catechol system at high pH is remarkable. As shown in Fig. 2, the curve relating E'_0 to pH undergoes two inflections. As noted by Hall, Preisler, and Cohen (16), when the value of $-d^2E'_0/dpH^2$ becomes negative, the dissociation causing

the change is assignable to the reductant; when the value becomes positive, the dissociation is that of the oxidant. Therefore the offset found here in the E'_0 : pH curve, implying two inflections of opposite orientations, could be accounted for if we assume one dissociation exponent of catechol and, at a somewhat higher pH, one dissociation exponent of orthoquinone. This would demand of orthoquinone structural peculiarities. The dissociation of a hydrogen from the benzene nucleus or the addition of a hydroxyl ion might be imagined. Since the present data concern physical measurements, we shall proceed with the usual, *formal* assumptions which lead to Equation 8 containing constants defined as follows:

$$\frac{(\text{H}^+) [\text{HRed.}]^-}{[\text{H}_2\text{Red.}]} = K_{r_1} \quad \frac{(\text{H}^+) [\text{Red.}]^-}{[\text{HRed.}^-]} = K_{r_2} \quad \frac{(\text{H}^+) [\text{Ox.}^-]}{[\text{HOx.}]} = K_o$$

$$[S_r] = [\text{Red.}]^- + [\text{HRed.}]^- + [\text{H}_2\text{Red.}]$$

$$[S_o] = [\text{HOx.}] + [\text{Ox.}]^-$$

In deriving Equation 8, it is assumed that the second dissociation constant of catechol is negligibly small.

$$E_h = E_0 + 0.03006 \log \frac{[S_o]}{[S_r]} + 0.03006 \log \frac{(\text{H}^+)^3 + K_{r_1} (\text{H}^+)^2}{K_o + (\text{H}^+)} \quad (8)$$

The curve of Fig. 2 is drawn to correspond with this equation when $E_0 = 0.792$, $K_{r_1} = 1.79 \times 10^{-9}$, and $K_o = 6.31 \times 10^{-11}$.

It is recognized that the experimental data may be at fault, since, in the region of pH where this inflection of the curve occurs, the oxidant is very unstable. However, the data obtained at any one pH value showed good agreement among values of E'_0 calculated for different percentages of reduction. In order to obtain a check on the validity of the data, we have made a similar study of the system benzohydroquinone-benzoquinone in this same pH range. The data are presented in Fig. 2, Curve F. The curve drawn to fit the data is expressed by Equation 6 in which $E_0 = 0.6955$, $K_{r_1} = 6.61 \times 10^{-10}$, and $K_{r_2} = 5.25 \times 10^{-13}$. The results obtained are in complete accord with the expected behavior of this system and give no evidence that the unstable paraquinone behaves as the orthoquinone does.

In the majority of cases the data presented for the catechol system in the alkaline range were obtained by running a solution of the reductant in conductivity water against a buffered solution of ferricyanide. In the case of the benzohydroquinone system this same procedure was followed and in addition each point was checked by running an equimolecular mixture of quinone and hydroquinone in conductivity water against a plain buffer solution. Both these procedures were found to give the values obtained by

TABLE VII
l-3,4-Dihydroxyphenylalanine. Relation of E'_0 and of $-\Delta E_h/\Delta t$ to pH
Temperature 30.0°.

Oxidizing agent	pH	E'_0	E_0 calculated assuming $-\Delta E'_0/\Delta \text{pH} = 0.0601$	$-\Delta E_h/\Delta t$ <i>mv. per sec.</i>
Ce(SO ₄) ₂	0.028	0.802	0.804	0.08
"	0.919	0.753	0.808	0.15
"	1.295	0.726	0.804	0.28
"	1.295	0.725*	0.803	0.27
"	1.832	0.686	0.797	0.23
"	1.832	0.688†	0.798	0.30
"	1.887	0.685	0.798	0.33
Br ₂	2.670	0.635	0.795	1.3
"	3.398	0.589	0.793	2.2
Ce(SO ₄) ₂	4.130	0.543	0.791	0.28
"	4.694	0.511	0.793	0.81
Br ₂	6.076	0.435	0.798	
"	6.702	0.393	0.796	35.0
Fe(CN) ₆ ⁼	7.664	0.326	0.787	64.0

* Synthetic racemic sample.

† Unrecrystallized sample.

the employment of the usual technique described earlier. Changes in pH due to dilution of the buffer were determined and the potentials were corrected accordingly.

Table VII shows the relation of E'_0 and of $-\Delta E_h/\Delta t$ to pH for the system whose reductant is the amino acid, 3,4-dihydroxyphenylalanine. One run was made with a synthetic *dl* preparation at pH 1.295. The value obtained agreed well with that given by the levo preparation at the same pH. This is in agreement

TABLE VIII
Relation of E'_0 and of $-\Delta E_h/\Delta t$ to pH

Temperature 30.0°.

pH	Protocatechuic acid				Ethyl ester of protocatechuic acid				Gentisinic acid				Ethyl ester of gentisinic acid				Pyrogallol				Gallic acid			
	E'_0		$-\frac{\Delta E_h}{\Delta t}$		E'_0		$-\frac{\Delta E_h}{\Delta t}$		E'_0		$-\frac{\Delta E_h}{\Delta t}$		E'_0		$-\frac{\Delta E_h}{\Delta t}$		E'_0		$-\frac{\Delta E_h}{\Delta t}$		E'_0		$-\frac{\Delta E_h}{\Delta t}$	
		E_0^*		<i>mv. per sec.</i>		E_0^*		<i>mv. per sec.</i>		E_0^*		<i>mv. per sec.</i>		E_0^*		<i>mv. per sec.</i>		E_0^*		<i>mv. per sec.</i>		E_0^*		<i>mv. per sec.</i>
0.028	0.883	0.884	0.10	0.883	0.885	0.03	0.795	0.796	4.1	0.792	0.794	0.09	0.715	0.716	60	0.807	0.809	66			0.807	0.809	66	
0.919	0.829	0.884	0.09	0.830	0.885	0.03	0.740	0.795	2.2	0.738	0.793	0.08	0.652	0.707	26	0.742	0.797	33			0.742	0.797	33	
1.289	0.806	0.884	0.07	0.807	0.885	0.03							0.638	0.715	37	0.724	0.801	37			0.724	0.801	37	
1.295							0.714	0.792	8.3	0.715	0.793	0.15												
1.522	0.794	0.886	0.14	0.792	0.883	0.03																		
1.832	0.770	0.880	0.11																					
1.887	0.768	0.881	0.11	0.771	0.884	0.03	0.674	0.788	2.8	0.680	0.793	0.20	0.603	0.716	25	0.674	0.788	41			0.674	0.788	41	
Average..		0.883			0.884			0.793			0.793			0.713			0.799					0.799		

* Calculated, assuming $-\Delta E'_0/\Delta pH = 0.0601$.

with the results obtained with the isomers of epinephrine. The values for $-\Delta E'_0/\Delta pH$ are fairly close to 0.0601 at pH values greater than 2. At lower pH values there is a deviation in the direction of a "0.09 slope." This can be seen from the increase in the calculated E'_0 values in this pH range. It is to be expected that within this pH range a shift in the E'_0 : pH curve could occur due to the difference in the dissociation constants of an ionizable group, either carboxyl or amino, of the reductant and the oxidant. The shift towards a "0.09 slope" indicates that the ionizable group of the oxidant is stronger than that of the reductant. Blix (4)

TABLE IX

Relation of E'_0 and of $-\Delta E_h/\Delta t$ to pH for Several Pressor Substances
Temperature 30.0°.

pH	Adrenalone			Epinephrine			Norhomoepinephrine		
	E'_0	E_0^*	$-\frac{\Delta E_h}{\Delta t}$	E'_0	E_0^*	$-\frac{\Delta E_h}{\Delta t}$	E'_0	E_0^*	$-\frac{\Delta E_h}{\Delta t}$
			<i>mv. per sec.</i>			<i>mv. per sec.</i>			<i>mv. per sec.</i>
0.028	0.905	0.906	4.1	0.783	0.785	0.012	0.817	0.818	0.015
0.919	0.860	0.915	6.0	0.735	0.790	0.002	0.769	0.825	0.01
1.289				0.712	0.790	0.007	0.745	0.823	0.004
1.295	0.833	0.911	2.8						
1.887	0.791	0.905	7.4	0.676	0.789	0.013	0.708	0.821	
4.130				0.539	0.788	2.0			
7.664				0.306	0.767†	168.0			
Average.....		0.909			0.788			0.822	

* Calculated, assuming $-\Delta E'_0/\Delta pH = 0.0601$.

† Value excluded from the average.

found the carboxyl group of the oxidant of the homogentisic acid system to be more easily ionized than the same group in the reductant. The first obvious change in the E'_0 : pH curve for dihydroxyphenylalanine occurs between pH 1 and 2. This is in agreement with the pK' value, 2.26, assigned by Miyamoto and Schmidt (21) to one of the ionizable groups of this amino acid. The data obtained in this study are not accurate enough to enable us to assign an exact value to the dissociation constant.

In Table VIII are assembled data showing the relation of E'_0 and of $-\Delta E_h/\Delta t$ to pH for six compounds. With the exception

of the ethyl esters of protocatechuic and gentisinic acids all of these compounds are known to occur in plants. The slight decrease in the E_0 values at pH 1.887 for the compounds with a carboxyl group attached to the ring may be due to the effect of the difference in dissociation constants for this group in the reductant and oxidant as discussed in the case of dihydroxyphenylalanine. The fact that the ethyl esters show no such change substantiates this supposition.

Table IX contains values showing the relation of E'_0 and of $-\Delta E_h/\Delta t$ to pH for three, synthetic, pressor substances.

The normal potentials for all systems studied are summarized in Table X.

DISCUSSION

It is of interest to observe the relation between structure and free energy change. A comparison of the data on benzohydroquinone and catechol shows that catechol has a normal potential nearly 100 millivolts more positive than that of benzohydroquinone. The difference in normal potential between protocatechuic acid and gentisinic acid is of the same order of magnitude. This characteristic potential difference between the para and the ortho isomers is not confined to the quinones; Conant and Pratt (11) have shown the same for quinoimines. The normal potentials of epinephrine, 3,4-dihydroxyphenylalanine, and epinine are all very close to that of catechol. The various side chains apparently do not modify greatly the normal potential of catechol. These experimental facts are in agreement with the usual rule that compounds of similar chemical structures possess approximately the same normal potential. As an exception, however, norhomoepinephrine has a normal potential 30 millivolts more positive than that of catechol. Pyrogallol is a "stronger" reducing agent than catechol by reason of the substitution of a hydroxyl group. A difference of 79 millivolts exists between the two systems. Conant and Fieser (10) and Fieser (13) have reported similar results for the substitution of a hydroxyl group in other systems. These same workers have also shown that the substitution of a carboxyl group raises the normal potential. Among the compounds studied in the present investigation, the following three pairs, pyrogallol-gallic acid, catechol-protocatechuic acid, and

benzohydroquinone-gentisinic acid, show the effect of such a substitution. The effect of the carboxyl group is to raise the normal potential 86, 91, and 97 millivolts respectively. The potential of

TABLE X
Summary of E_0 and $-\Delta E_h/\Delta t$ for All Compounds
Temperature 30.0°.

Reductant	E_0	$-\frac{\Delta E_h}{\Delta t}$ at pH 1.3
HO HO  , catechol.....	0.792	0.011
R-CHOH·CH ₂ ·NH·CH ₃ , epinephrine.....	0.809	0.03
R-CH ₂ ·CH·NH ₂ ·COOH, dihydroxyphenylalanine.....	0.800	0.28
R-CH ₂ ·CH ₂ ·NH·CH ₃ , epinine.....	0.788	0.007
R-CHOH·CH·NH ₂ ·CH ₃ , norhomoepinephrine.....	0.822	0.004
R-CO·CH ₂ ·NH·CH ₃ , adrenalone.....	0.909	2.8
R-COOH, protocatechuic acid.....	0.883	0.07
R-COOC ₂ H ₅ , ethyl ester of protocatechuic acid...	0.884	0.03
HO HO  , pyrogallol.....	0.713	37.0
HO HO  COOH, gallic acid.....	0.799	37.0
HO  OH, gentisinic acid.....	0.793	8.3
COOH		
HO  OH, ethyl ester of gentisinic acid.....	0.793	0.15
COOC ₂ H ₅		
HO  OH, benzohydroquinone.....	0.696	

R = 3,4-dihydroxyphenyl.

adrenalone was found to be 118 millivolts higher than that of catechol, presumably because of the carbonyl group attached to the benzene ring. It appears that the effect of substitution of one carboxyl group is to produce an increase of normal potential of

the same order of magnitude, provided the parent compounds are comparable in other respects.

An idea of the rapidity with which the oxidant of any compound decomposes can be obtained from the data listed in Tables VI to X under the column " $-\Delta E_h/\Delta t$," *i.e.*, the change of potential in millivolts per second. These data were obtained, either by following the drift of potential with time after stopping the flow of the mixture, or directly from the potentials at the various electrodes during active flow of the mixture. Changes in the initial concentration of oxidant at fixed pH caused but little change in the value of $-\Delta E_h/\Delta t$ when the percentage of oxidation was 50 per cent or less. At higher percentages of oxidation the value of $-\Delta E_h/\Delta t$ tended to increase in some instances, though the relationship remained linear. Values reported are for an approximately equimolecular mixture of oxidant and reductant. A change of potential of about 9 millivolts corresponds to the disappearance of half the amount of oxidant originally present in an equimolecular mixture. Hence the so called "half life" of an oxidant, in seconds, can be readily obtained by dividing 9 millivolts by the given value of $-\Delta E_h/\Delta t$. For example, the value of $-\Delta E_h/\Delta t$ for epinephrine at pH 7.66 and 30° is 150 millivolts per second. Therefore the "half life" of the oxidant is 0.06 second. The velocity constant, k , for the decomposition of any oxidant can be calculated from the following equation obtained by combining Equations 4 and 5. Since $-\Delta E_h/\Delta t$ is expressed in Tables VI to X as millivolts per second, instead of volts per second, the factor 1000 is used in Equation 9.

$$k = nF/1000 RT (-\Delta E_h/\Delta t) \quad (9)$$

In the case of epinephrine, cited above, $k = 11.5$.

The orthoquinones are usually more unstable than the paraquinones. As an exception, the oxidant of gentisinic acid is more unstable than the oxidant of protocatechuic acid. In general the substitution of an ionizable side chain increases the inherent instability of a quinone as noted by Nef (22), who found it difficult to prepare the oxidants of several hydroquinone carboxylic acids. This effect is seen on comparing the results for systems in which an acid and its ethyl ester respectively are the reductants. Although there is but an insignificant difference in the normal

potentials, there is a marked difference in the stabilities of the oxidants.

Attempts were made to develop *quantitative* relations between the observed values of $-\Delta E'_0/\Delta t$ and pH. No simple relation was discovered. Qualitatively it may be said that, with the exception of some anomalous effects in very acid solution, the instability of each oxidant increases rapidly with increase of the pH value of the solution. Also at 37.4° the oxidant of catechol is about twice and that of epinephrine about thrice as unstable as at 30°.

On p. 705 are mentioned the normal potentials of the catechol system at different temperatures. A comparable fall of potential with increase of temperature was noted for the epinephrine system. We have not attempted accurate estimates of the temperature coefficients of the normal potentials of the several systems because there are lacking certain basic data for corrections to an acceptable basis. However, neglect of accurate temperature coefficients will not be significant in the following discussion.

The naturally occurring derivatives of catechol and of benzo-hydroquinone are reductants of systems having characteristic potentials which are distinctly positive to that of the system of which 2,6-dichlorophenol indophenol is the oxidant. This dye is rapidly and completely reduced by most living cells. Since there is no reason to believe that either this dye or any one of the quinones requires specific reducing agents or specially catalyzed reducing processes, the free energy data seem sufficient for the following conclusion. Cells having systems capable of completely reducing 2,6-dichlorophenol indophenol must be capable of maintaining the systems now under discussion extensively in the reduced state. The argument can be elaborated by consideration of the conduct in cells or tissues of other indicators or of other naturally occurring systems such as the hemoglobin-methemoglobin system.

Conversely, the fact that several of the reductants of the systems now under consideration are found in cells or in their immediate environment, and at levels of pH which would ordinarily enhance the ease of their oxidation by oxygen, may be used in support of the impression, gained from various sources, that the level of the generalized intensity factor of the free energy of cellular oxidation and reduction is well below that required to maintain in a tem-

porary state of equilibrium any appreciable amount of the quinones in question. This conclusion is strengthened when we consider the apparent protective action of cells toward the reductants in question in relation to the peculiarities arising from the inherent instability of the oxidants. The significance of the latter fact can best be shown by noting the errors which have arisen in efforts to evaluate the oxidation-reduction potentials of the epinephrine and related systems by their behavior toward dyes of known characteristics. Kendall (18) reported that 2,6-dibromophenol indophenol was reduced by epinephrine and by adrenalone at pH 7.4. Blix (4) reported this same dye, as well as *o*-cresol indophenol, to be partly reduced by epinephrine at pH 7.0. However, at pH 7.0, for example, equivalent amounts of epinephrine and 2,6-dichlorophenol indophenol² would react so that *initially* about 0.1 per cent of the dye would be reduced and the equivalent amount of the epinephrine would be oxidized. The oxidized epinephrine would quickly decompose and to maintain the flow of energy further reaction would occur. By the constant withdrawal of the oxidized epinephrine through decomposition the reaction would go to completion with complete reduction of the dye. Neglect of the decomposition and use of the ordinary interpretation of indicator conduct leads to the false conclusion that the potential of the epinephrine system is negative to that of the dye systems; whereas, in fact, the opposite is true. We have found that when equivalent amounts of epinephrine and 2,6-dichlorophenol indophenol are dissolved in a buffer solution of pH 7.0 and in the absence of oxygen, the dye is completely decolorized within about 1 hour. The red decomposition product usually obtained upon oxidizing epinephrine does not appear. Potentials measured during the course of the reaction progressively decline, lie within the region characteristic of the dye, and are not those to be expected if the dye system alone were influencing the electrode. It is possible to restore the original color of the dye to practically full intensity by the addition of an equivalent amount of ferricyanide. The dye is then slowly decolorized again. This may be repeated. In short, 1 equivalent of epinephrine is capable of decolorizing 4 equivalents of the dye. If epinephrine be first

² The potentials characteristic of the dichloro- and dibromoindophenols are nearly the same.

completely oxidized by ferricyanide with the formation of the usual red product and then dye be added in amount equivalent to the initial epinephrine, decolorization takes place and the same number of equivalents are used as in the experiment previously mentioned. These facts indicate that decolorization of the dye is due to reduction, although the absence of the red decomposition product of oxidized epinephrine, the peculiar conduct of electrodes, and the reduction of 4 equivalents of dye suggest side reactions. Several types of these might be postulated from the known conduct of analogous compounds. Undoubtedly there are concerned processes similar to those outlined by Raper (24) in his report upon the oxidation of dihydroxyphenylalanine to melanin.

Chambers, Cohen, and Pollack (5) have noted that the use of an oxidation-reduction indicator system, the reductant of which is unstable (as is one reductant of Janus green), is subject to the serious objection that such an indicator may be completely reduced by another more electropositive system. We now suggest that care be used in the interpretation of results obtained upon applying *stable* indicator systems to systems with unstable components. Since many biologically important oxidation-reduction systems are of this category, there will arise the question whether the reduction of a dye by a cell is indicative of a level of oxidation-reduction intensity pertaining to a true equilibrium state. Results with such situations should be scrutinized with especial care when increasingly more negative indicator systems require longer times for reduction, since such a set of relations is what may be expected if the dye is being reduced by a more positive system with unstable oxidant. Of this pattern are most of the results of the microinjection studies.

It is interesting to note that the systems of which *l*- and *d*-epinephrine are the reductants show no significant difference in potential. It is to be expected that the free energies of optical isomers will be identical, since they are produced in equal quantities when synthesized *in vitro*. While the precision of the present investigation is inadequate to give this proposition a severe test, the results are not inconsistent with the prediction and constitute what we believe to be the first recorded test. These results also show that the pharmacological action of a compound is not dependent on the free energy level of its system since this level is the same in both the cases under consideration, while the difference in

pharmacological action is pronounced. This statement must not be construed to imply that the oxidation-reduction intensity of the environment will not affect the pharmacological action of a compound.

It is well known that the intravenous injection of epinephrine into an animal causes a pronounced rise of blood pressure which is, however, of short duration. Can this transient action be connected in any way with the oxidation and consequent destruction of this hormone at the site of its action? The fact that ephedrine, which contains no orthohydroxy groups and so should be less easily oxidized and destroyed, has a prolonged effect on blood pressure is suggestive, though it must be remembered that this difference may be due to a difference in the site of action. Such a mechanism, however, would demand either that the tissues where epinephrine is synthesized or stored have a reducing intensity widely different from those tissues on which it exerts its action, or else epinephrine is liberated from a precursor at the site of action. The existence of epinephrine precursors in the body has been reported by Kendall (19) and by Annau, St. Huszák, Svrbely, and Szent-Györgyi (1).

The mechanism of the oxidase of the higher plants has been assumed to be the formation of an organic peroxide from the dihydroxy group characteristic of catechol. Szent-Györgyi (27) has suggested that an orthoquinone is formed. If any of the compounds studied here are to act even at low pH values as cyclic catalysts by the formation of orthoquinones, either the process must be a comparatively rapid one, or else some mechanism to stabilize the orthoquinone is involved; otherwise an appreciable destruction of the catalyst would occur.

The oxidation of many of these compounds results in the formation of colored products. These colored products are not the quinones that are first formed. This is apparent from the fact that color intensity is still at a maximum when electrode potentials indicate that all quinone has disappeared. This has a bearing upon the usual colorimetric methods of analysis.

SUMMARY

An apparatus for the study of the oxidation-reduction potentials of unstable systems is described. With this apparatus thirteen systems, the stable reductants of which are catechol, benzohydro-

quinone, or their derivatives, were studied at 30° and various pH values. The curve relating pH and the potentials of the half reduced solutions, E'_0 , for each system has a slope $-\Delta E'_0/\Delta \text{pH} = 0.06$ in acid solution. The instability of the oxidants of most of these systems increased to such an extent in the alkaline range that only two, catechol and hydroquinone, were amenable to study in solutions with pH values greater than 8.0. The data obtained for the benzohydroquinone system were in accordance with theoretical expectations, 2 hydrions dissociating from the reductant as pH increased so that $-\Delta E'_0/\Delta \text{pH}$ approached zero. The dissociation constants were assigned the values $K_{r_1} = 6.61 \times 10^{-10}$ and $K_{r_2} = 5.25 \times 10^{-13}$. The catechol system was peculiar in that the data for the alkaline range required the assumption of a dissociable hydrion of the oxidant and the dissociation of only 1 hydrion from the reductant. The values for these dissociation constants are $K_{r_1} = 1.78 \times 10^{-9}$ and $K_o = 6.31 \times 10^{-11}$.

The relation of structure and free energy is discussed. The results are in general agreement with those found for other systems. The systems of which the isomers of epinephrine are the reductants are found to possess the same normal potential.

The change of potential with time is used as the criterion of the instability of an oxidant. In general the data show that the substitution of an ionizable side chain markedly increases the inherent instability of quinones and that the oxidants of all systems become more unstable as pH increases.

The potentials of the naturally occurring systems were found to be so positive that living tissues under normal physiological conditions can keep them practically completely in the reduced state. The reductants of these systems are thereby protected from a virtually destructive oxidation.

The reduction of an oxidation-reduction indicator by a more positive but unstable system is noted. It is emphasized that results obtained by means of oxidation-reduction indicators on unstable systems or on biological material where such systems are present must be interpreted with great care.

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